**SUPPLEMENTARY TABLE S1. Analytical sensitivity of IgG anti-HEV ELISA\***

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Assayed** | **Pre-assay** |  | **Run 1** |  |  | **Run 2** |  |
| **material** | **concentration** |  | **S/COmean** | **CV** |  | **S/COmean** | **CV** |
| **WHO** | **6.25 U/mL** |  | 6.38 | 11% |  |  |  |
| **Reference** | **4.00** |  |  |  |  | 7.00 | 8% |
| **Reagent** | **3.13** |  | 3.15 | 9% |  |  |  |
| **for**  | **2.00** |  | 2.28 | 13% |  | 3.76 | 5% |
| **Anti-HEV** | **1.00**† |  | 1.28 | 53% |  | 1.78 | 10% |
|  | **0.50** |  | 0.49 | 4% |  | 0.81 | 6% |
|  | **0.25** |  | 0.25 | 4% |  | 0.35 | 6% |

\* runs performed with a single ELISA lot and quadruplicate aliquots of each

concentration, 27 days apart by a single operator.

† concentration in the ELISA was 0.091 U/mL (10 µL of 1.0 U/mL added to 100 µL

of specimen diluent).

**SUPPLEMENTARY TABLE S2. Reagent oligonucleotides**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Purpose** | **Region\*** | **Function** | **Span**† | **Sequence 5’ → 3’**‡ | **Product** | **Reference** |
| qRT-PCR | HEV ORF3 | forward | 5261-5278 | GGTGGTTTCTGGGGTGAC | 70 nt§ | Jothikumar et al. 26 |
| (detect & |  | reverse | 5330-5313 | AGGGGTTGGTTGGATGAA |  |  |
| quantify) |  | probe | 5284-5301 | [FAM]TGATTCTCAGCCCTTCGC[NFQ-MGB] |  |  |
|  | MS2 | forward | 289-314 | TGGCACTACCCCTCTCCGTATTCACG | 99 | Rolfe et al. 23 |
|  |  | reverse | 387-366 | GTACGGGCGACCCCACGATGAC |  |  |
|  |  | probe | 330-358 | [JOE]CACATCGATAGATCAAGGTGCCTACAAGC[NFQ-MGB] |  |  |
| RT → | HEV ORF1 | outer forward | 32-55 | AGGCCCAYCAGTTYATWAAGGCTC | 681 | Dong et al. 27 |
| nested PCR |  | outer reverse | 713-691 | TASCCWGCACTAGWGTCMCCCTC |  |  |
| (sequence) |  | inner forward | 56-79 | CTGGCRTYACWACTGCYATTGAGC | 576 |  |
|  |  | inner reverse | 631-610 | TRCCRGGKGGKARCAGSACCTC |  |  |
|  |  | outer forward | 56-79 | CTGGCATYACTACTGCYATTGAGC | 418 | Wang et al 28;  |
|  |  | outer reverse | 473-451 | CCATCRARRCAGTAAGTGCGGTC |  | unpublished‡ |
|  |  | inner forward | 104-124 | CTGCCYTKGCGAATGCTGTGG | 286 |  |
|  |  |  |  | CTGCCtTgGCGAATGCTGTGG‡  |  |  |
|  |  | inner reverse | 389-367 | GGCAGWRTACCARCGCTGAACATC |  |  |
|  |  |  |  | GGCAGtaTACCAgCGCTGAACATC‡  |  |  |
|  | HEV ORF2 | outer forward | 6296-6318 | CCGACAGAATTGATTTCGTCGGC | 308 | Zhai et al. 29; |
|  |  | outer reverse | 6603-6581 | CCGTAcGTrGtCTGrTCrTACTC‡  |  | unpublished‡ |
|  |  | inner forward | 6347-6369 | GTTGTCTCGGCCAATGGCGAGCC | 236 |  |
|  |  | inner reverse | 6582-6560 | TCGGCGGCGGTGAGAGArAGCCA‡  |  |  |

\* ORF, open reading frame; HEV ORF3 region also includes overlapping 5’ segment of ORF2

† HEV spans, nucleotide-positions per GenBank accession [M74506](https://www.ncbi.nlm.nih.gov/nuccore/M74506.1); MS2 spans, [NC\_001417](https://www.ncbi.nlm.nih.gov/nuccore/NC_001417)

‡ brackets, label and quencher respectively attached to 5’ and 3’ end of each probe; lower case, residues respectively modified (Forman & Ticehurst, unpublished data) from inner primers for HEV ORF1 28 and from reverse-primers MJ-D EAP and MJ-D IAP for ORF2 29

§ nt, nucleotides (length)